11. (**Amended**) The method of any of claims 1-4[1-8], wherein the *hedgehog* polypeptide is modified with one or more fatty acid moieties.

REMARKS

Claims 1-21 constitute the pending claims in the present application. Applicants note that claims 5-10 and 12-21 have been withdrawn from consideration, and claims 1-4 and 11 were elected with traverse. Applicants will cancel non-elected claims upon indication of allowable subject matter. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action. Applicants respectfully request reconsideration in view of the following remarks.

1. Claims 1-4 and 11 are rejected under 35 U.S.C. 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the prior office action states that "the instant specification fails to provide information that would allow the skilled artisan to practice the instant invention without undue experimentation." Applicants traverse this rejection.

The prior office action contends that since the scope of the claims is very broad and the specification fails to provide guidance for selecting the lipophilic modifications used to practice the methods of the invention, it would require undue experimentation to practice the invention. Applicants disagree with this characterization, and maintain that the specification provides extensive guidance concerning lipophilic modified *hedgehog* polypeptides, and furthermore that the specification provides methods such that one of skill in the art could identify which lipophilic modified polypeptides would be useful in practicing the claimed methods without expending any more energy than is normally required in the art.

Applicants describe in great detail the sequence and functional characteristics of *hedgehog* polypeptides. This includes discussion of *hedgehog* processing, and of the *hedgehog* signaling pathway (page 2, line 32-page 3, line 8; page 23, line 19-page 25, line 16). Based on

the disclosure, one of skill in the art can readily envision the *hedgehog* polypeptides modified and employed in the methods of the present invention. Additionally, one can readily envision the structural and functional characteristics of *hedgehog* polypeptides, the *hedgehog* signaling pathway, and the role of *hedgehog* in a variety of developmental contexts.

Applicants have additionally provided extensive discussion of the potential lipophilic modifications utilized to practice the claimed methods. This description provides many illustrative examples, and also details exemplary portions of the *hedgehog* polypeptide that may be derivatized to practice the invention (page 3, lines 9-16; page 25, line 17-page 26, line 24). Furthermore, the disclosure provides detailed guidance for chemically coupling a lipophilic moiety or moieties to polypeptides (page 26, line 25-page 29, line 37). Such methods for chemically coupling lipophilic moieties are commonly practiced in the art. Accordingly, Applicants contend that the practice of chemically coupling lipophilic moieties to polypeptides requires nothing more than routine experimentation.

In further support of the enablement of the claimed subject matter, Applicants have provided examples demonstrating the efficacy of a myristoylated N-terminal fragment of *Sonic hedgehog* in a malonate striatal lesion model (Examples 5-7). Example 7 provides additional examples of hydrophobically modified *hedgehog* polypeptides which display activity greater than unmodified Shh in the malonate striatal lesion model.

Applicants contend that the specification contains extensive description and discussion of *hedgehog* polypeptides, *hedgehog* signaling, and lipophilic moieties. The disclosure further reviews the routine methods practiced in the art to couple lipophilic moieties to polypeptides. Given that the construction of lipophilic modified *hedgehog* polypeptides requires nothing more than routine experimentation, the only remaining question is whether one of skill in the art can recognize which of these derivatives are useful for practicing the invention. Applicants point out that Examples 5-7 demonstrate that one of skill in the art can readily evaluate the efficacy of any derivatized polypeptide. Furthermore, Examples 1-4 present several neuronal cell culture models in which the activity of the derivatized polypeptides can be readily evaluated.

Given the disclosure of the present application, coupled with the high level of skill in the art, the efficacy of any of a number of lipophilic modified *hedgehog* polypeptides can be readily

evaluated without undue experimentation. This is the standard under MPEP 2164.08(b) which states that "[t]he presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled. The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art." Applicants have met this burden and maintain that the claims are enabled throughout their scope. Reconsideration and withdrawal of the rejection is requested.

2. Claims 1-4, and 11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for allegedly failing to specifically point out and distinctly claim that which applicants regard as their invention. Applicants respectfully traverse this rejection.

Applicants define the *hedgehog* polypeptides of the invention in great detail. "The *hedgehog* therapeutic preferably is a polypeptide including at least a bioactive extracellular portion of a *hedgehog* polypeptide, e. g., including at least 50, 100, or 150 amino acid residues of an N-terminal half of a *hedgehog* polypeptide. In a preferred embodiment, the *hedgehog* portion includes at least a portion of the *hedgehog* polypeptide corresponding to a 19 kd fragment of the extracellular domain of a *hedgehog* polypeptide. In a preferred embodiment, the *hedgehog* portion has an amino acid sequence at least 60, 75, 85, 95 or 100 percent identical with a *hedgehog* polypeptide of any of SEQ ID Nos. 10-18 or 20. The *hedgehog* portion can be encoded by a nucleic acid which hybridizes under stringent conditions to a nucleic acid sequence of any of SEQ ID Nos. 1-9 or 19, e. g., the *hedgehog* portion can be encoded by a vertebrate *hedgehog* gene, especially a human *hedgehog* gene." (page 2, line 32-page 3, line 8) Furthermore, Applicants review in detail the *hedgehog* signaling pathway (page 23, line 19-page 25, line 16).

Similarly, Applicants describe in detail exemplary lipophilic modifications. "The *hedgehog* polypeptides of the present invention are modified by a lipophilic moiety or moieties at one or more internal sites of the mature, processed extracellular domain, and may or may not be also derivatized with lipophilic moieties at the N or C terminal residues of the mature polypeptide. In other embodiments, the polypeptide is modified at the C-terminal residue with a

hydrophobic moiety. In still other embodiments, the polypeptide is modified at the N-terminal residue with a cyclic (preferably polycyclic) lipophilic group. Various combinations of the above are also contemplated." (Page 3, lines 9-16). Additionally, exemplary lipophilic modifications are detailed throughout the specification, as are methods for chemically coupling such moieties to a polypeptide (page 25, line 17-page 26, line 24; page 26, line 25-page 29, line 37).

Finally, Applicants point out that contrary to the Examiner's contention that Applicants have failed to provide any examples demonstrating a lipophilic modified *hedgehog* polypeptide, Applicants have in fact provided Examples 5-7 which demonstrate the efficacy of a myristoylated-Shh in a malonate striatal lesion model.

Applicants contend that the specification provides extensive guidance concerning both *hedgehog* polypeptides and lipophilic modifications. Accordingly, one of skill in the art can readily recognize the metes and bounds of the claimed subject matter. Reconsideration and withdrawal of this rejection is requested.

3. Claims 1-4, and 11 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Ingham et al., in view of Pepinsky et al. Applicants respectfully traverse this rejection.

The Examiner has cited Pepinsky et al., and contends that the teachings of Pepinsky et al. combined with the teachings of Ingham et al., render the claimed subject matter obvious. In response, Applicants provide the declaration of Thomas Engber, Alphonse Galdes, and Nagesh Mahanthappa under 37 CFR 1.131 to "establish reduction to practice prior to the effective date of the reference, or conception of the invention prior to the effective date of the reference coupled with due diligence from prior to said date to a subsequent reduction to practice or to the filing of the application." (37 CFR 1.131(b)). Applicants contend that the declaration establishes that Applicants conceived and reduced to practice the subject matter of Pepinsky et al. prior to the publication of Pepinsky et al. Furthermore, the declaration asserts that Applicants exercised diligence in reducing the invention to practice following the publication of Pepinsky et al.

Applicants' declaration antedates the publication of Pepinsky et al. and removes Pepinsky et al. from consideration as prior art. Since the Examiner has already conceded that Ingham et al. alone is insufficient to render obvious the claimed subject matter, Applicants' declaration under 37 CFR 1.131 obviates the rejection under 35 U.S.C. 103(a). Reconsideration and withdrawal of this rejection are respectfully requested.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945.**

Respectfully Submitted,

Date: March 4, 2002

Matthew P. Vincent
Reg. No. 36,709

Customer No: 28120
Docketing Specialist
Ropes & Gray
One International Place
Boston, MA 02110

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Galdes et al.

Serial No: 09/325,602

Filed: June 3, 1999

For: Methods and Compositions for Treating Disorders Involving Excitotoxicity

Attorney Docket No. CIBT-P02-069

Art Unit: 1646

Examiner: M. Brannock

Assistant Commissioner of Patents
Washington, D.C. 20231

DECLARATION UNDER 37 CFR 1.131

Sir:

We, Thomas Engber, Alphonse Galdes, and Nagesh Mahanthappa hereby declare:

1. We are the named inventors of the pending claims of the patent application identified above and the inventors of the subject matter described in the patent application.

2. Prior to May 29, 1998, the listed publication date of Pepinsky et al., we had conceived the invention as described and claimed in the subject application in this country as evidenced by a slide presentation at an Internal Steering Committee Meeting (attached hereto as Exhibit 1). The data summarized on page 2 of Exhibit 1 demonstrates that we engineered and tested several different lipophilic modified forms of Sonic hedgehog, and showed that these lipophilic modified polypeptides were more potent than unmodified Sonic hedgehog in an in vitro assay. The slide presentation also predicted that such modified polypeptides could also be used in vivo in a Parkinson's Disease model. Furthermore, Exhibit 1, pages 3-8, demonstrates that we had conceived and reduced to practice the 6-OHDA lesion model for Parkinson's Disease, and had tested unmodified hedgehog polypeptides in this model. Accordingly, we had possession of both the lipophilic modified hedgehog polypeptides and the animal model for demonstrating their

increased potency in vivo, as well as plans to test the lipophilic modified hedgehog polypeptides in this in vivo model prior to May 29, 1998.

3. A few months after the presentation embodied in Exhibit 1, we initiated a study of the effects of supranigral microinjection of a modified Sonic hedgehog protein in 6-OHDA lesioned rats. Exhibit 2 is the protocol for this study, which was conducted at an outside preclinical testing services company in a NAFTA or WTO country. The study specifically examined the effects of myristoylated Sonic hedgehog in the 6-OHDA lesion model, and was initiated prior to May 29, 1998 (Exhibit 2, page 1). The experiments in this study were completed within a few months of the initiation thereof and prior to the effective filing date of the present application. Exhibit 3 is the final report of the study which demonstrates that a lipophilic modified hedgehog polypeptide functions with increased potency in vivo.

4. We assert that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true. We also understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 USC 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Thomas Engber — Dated: 2/28/02

Alphonse Galdes — Dated: 2/28/02

Nagesh Mahanthappa — Dated: _____

Attachments: Exhibits 1-3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Galdes et al.

Serial No: 09/325,602

Filed: June 3, 1999

For: Methods and Compositions for Treating Disorders Involving Excitotoxicity

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_____ Dated: _____
Thomas Engber

_____ Dated: _____
Alphonse Galdes

Nagesh Mahanthappa Dated: March 4, 2002

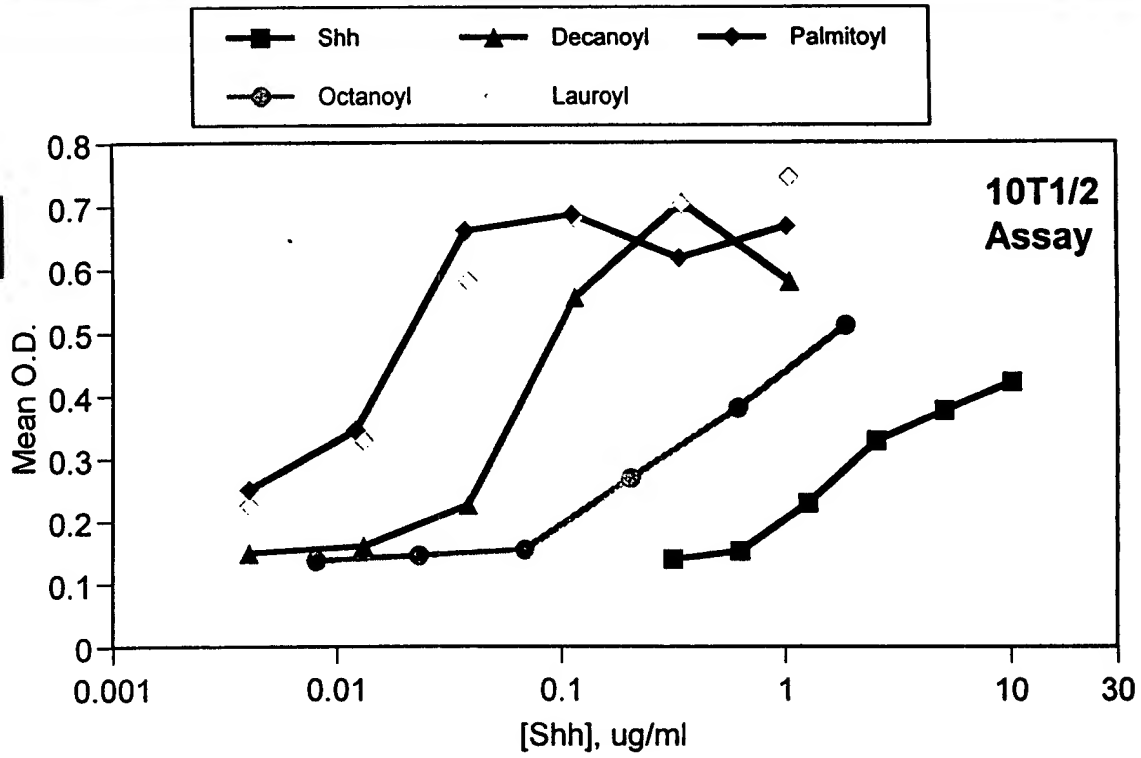
Attachments: Exhibits 1-3

Exhibit 1

Agenda for JMRC Presentation

- **Introduction**
- **Update on lipid-modified Shh**
- **Parkinson's Disease Models**
- **HH & Peripheral Nerve Biology**
- **Ptc-LacZ mice: pharmacology studies**
- **Other Animal Model Studies**

Protein Chemistry Update



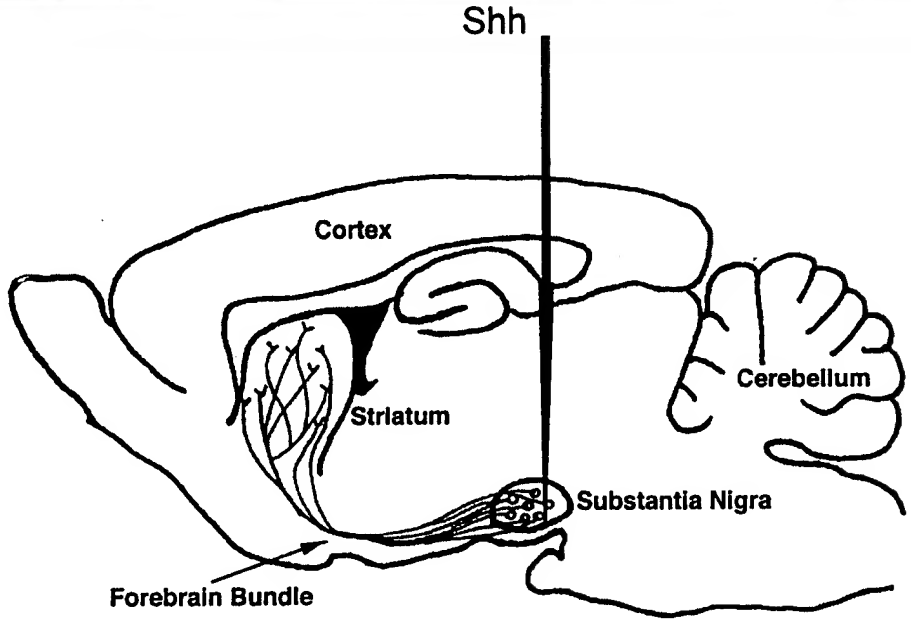
- in vitro acylation with acyl CoA
- Hydroxylamine
  - mono, Nterm derivative
- Dialysis to remove detergent
  - <C14 (myristol) , soluble protein

• increased in vivo potency?

•localized cns delivery?

myristoyl-Shh → **Striatal Lesion Parkinson's Disease Models**

Parkinson's Disease: 6-OHDA Lesion Rodent Model



6-OHDA Lesion

Protection Model

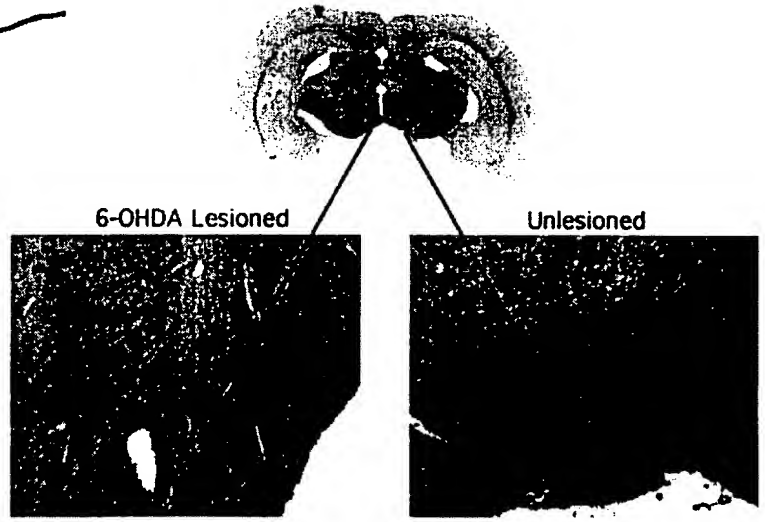
Treatment Model

Lesioned Side Unlesioned Side

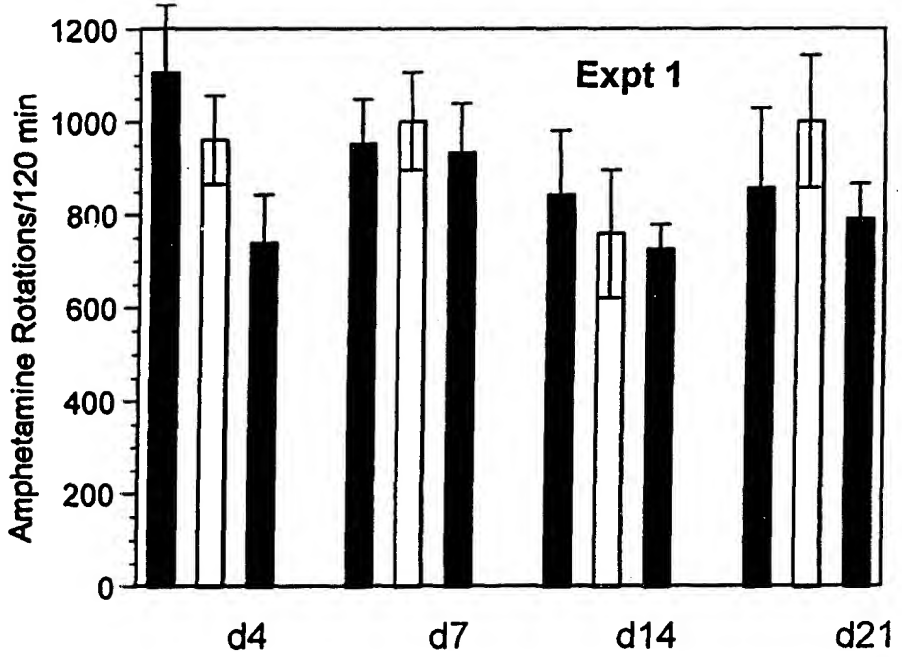
•Rotational Behavior

Amphetamine Apomorphine

- **DA & metabolite lev ls**
- **TH Immunohistochemistry**

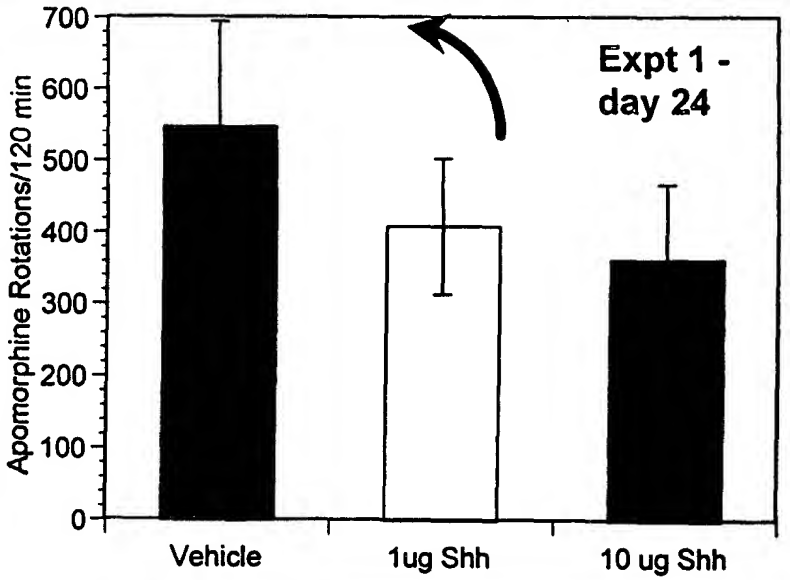


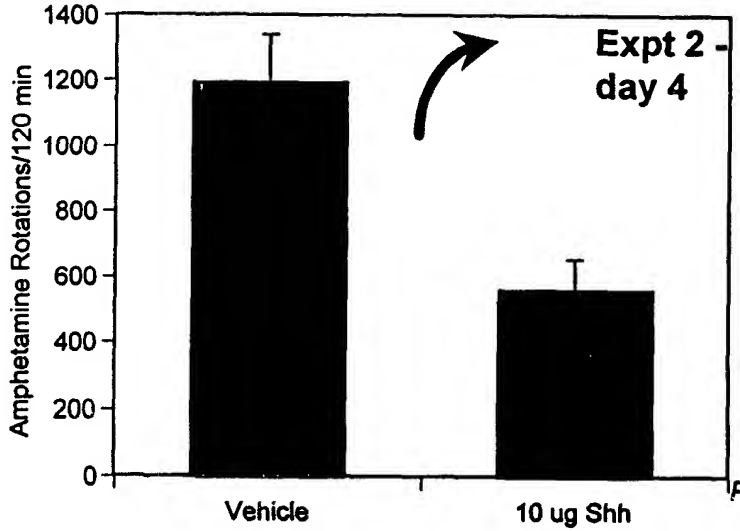
Parkinson's Disease 6-OHDA Lesion Model - Protection



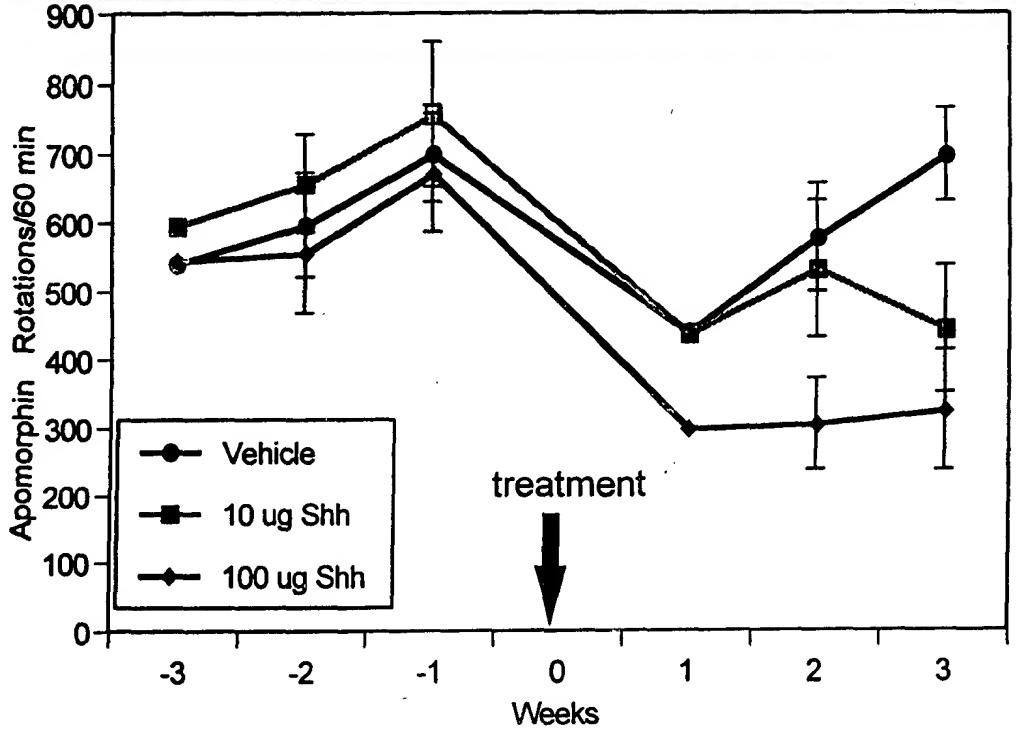
Vehicle
1ug Shh
10ug Shh

[Redacted]





Parkinson's Disease 6-OHDA Lesion Model - Treatment



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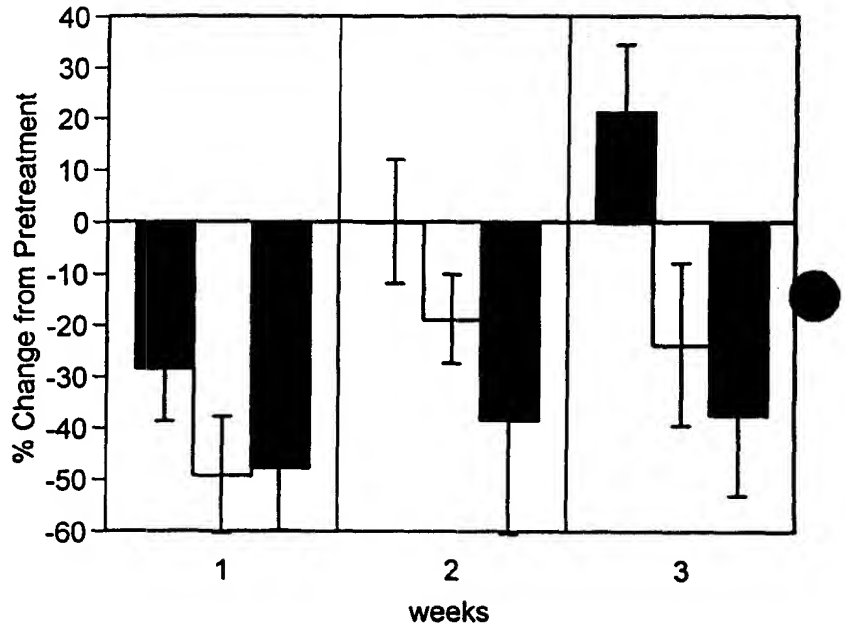
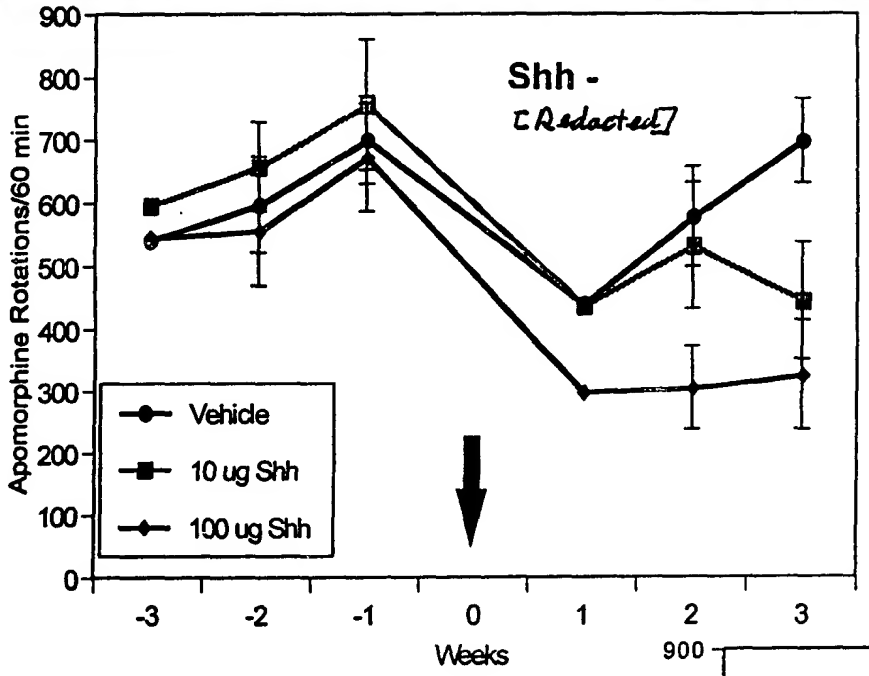
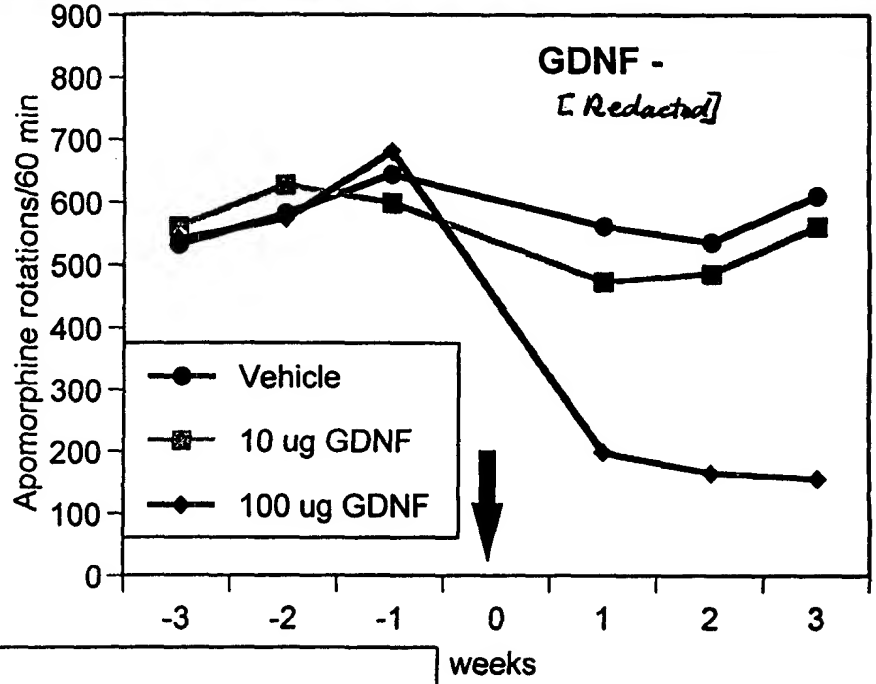
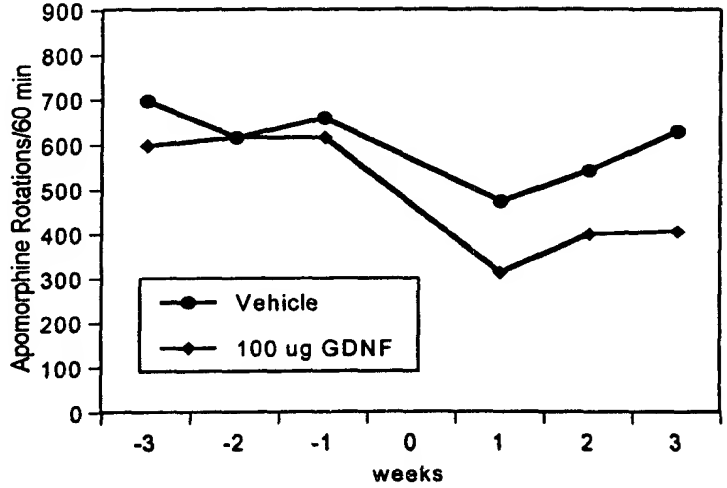


Parkinson's Disease 6-OHDA Lesion Model - Shh vs GDNF

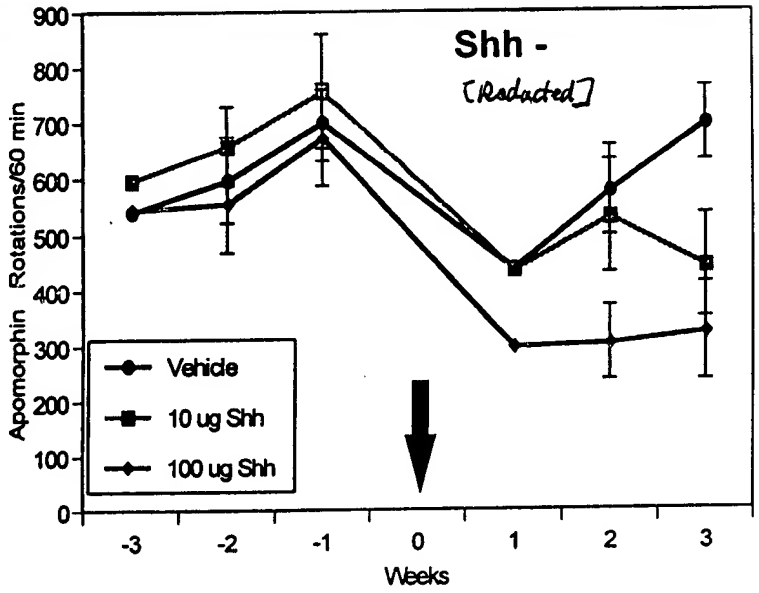


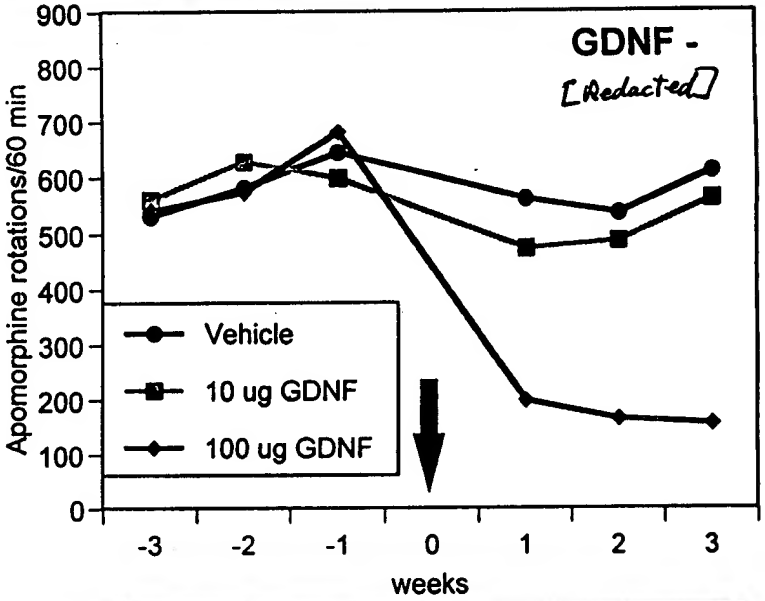


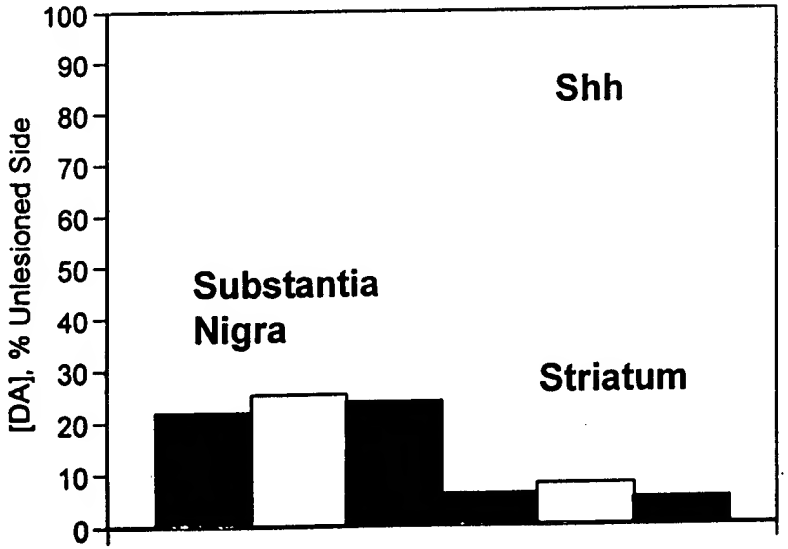


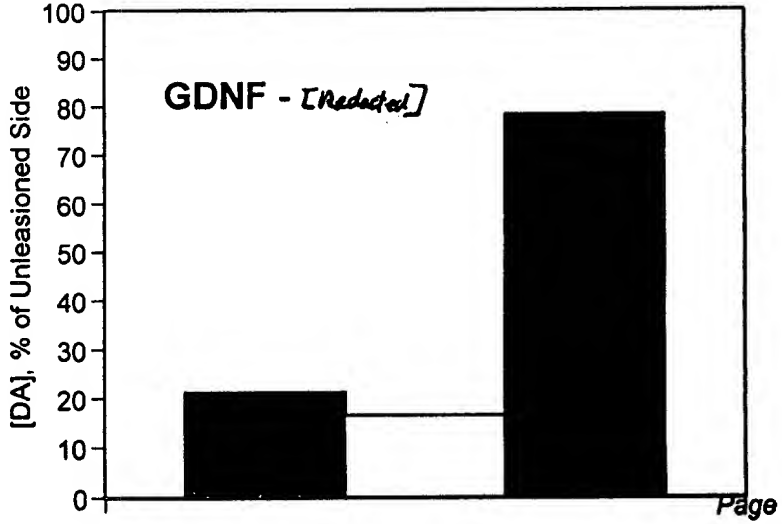
GDNF -
[Redacted]

Neurochemistry in 6-OHDA Lesion Model - Shh vs GDNF

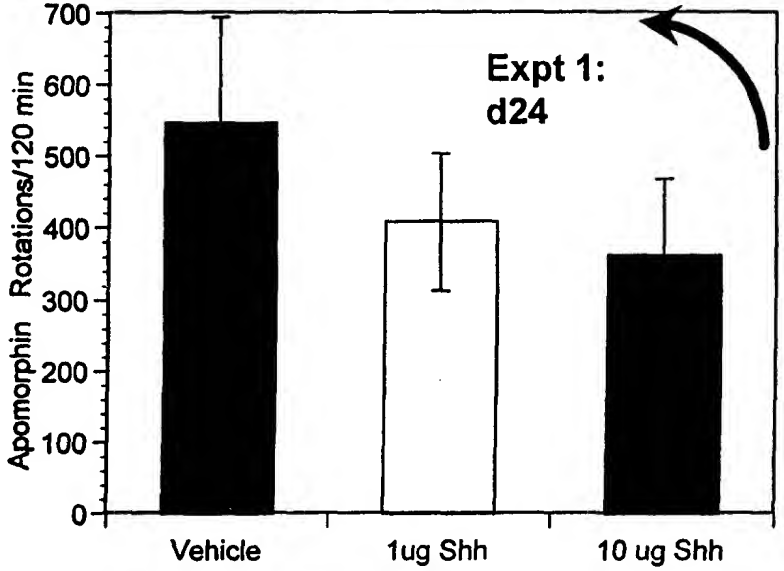


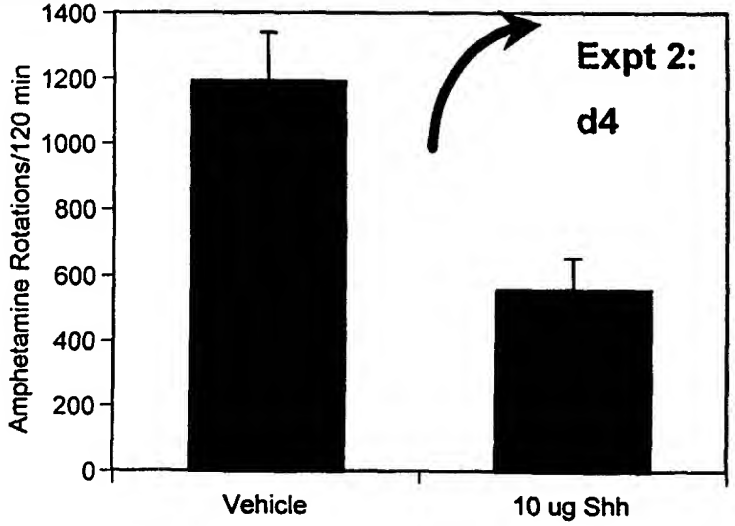


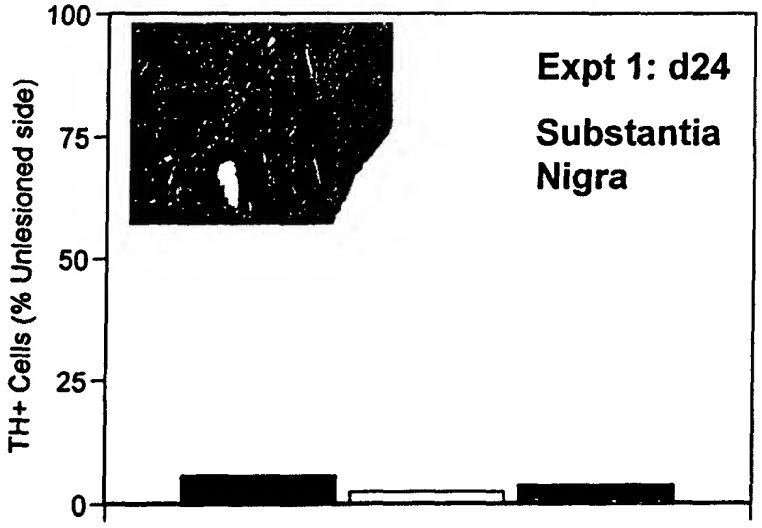




Immunohistochemistry in 6-OHDA Protection Model [Redacted]







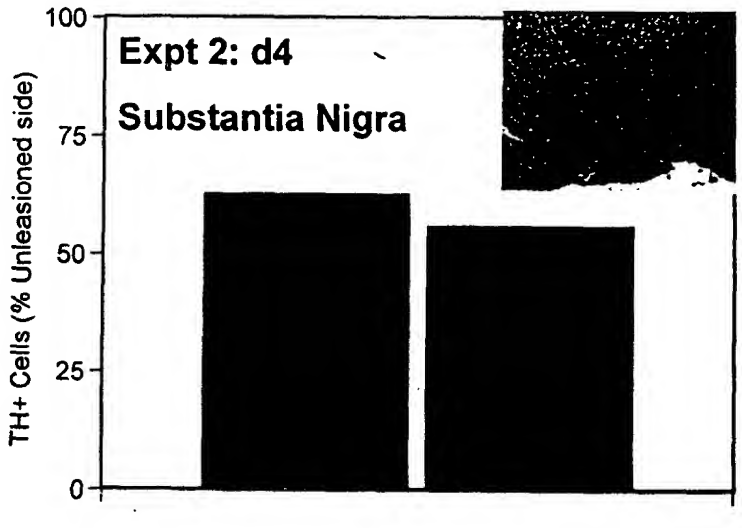


Exhibit 2

[Redacted]

STUDY PROTOCOL

Study Title: Effects of a Single Supranigral Microinjection of Modified Sonic Hedgehog Protein (Shh-M) in Unilateral 6-Hydroxydopamine Lesioned Rats

Project Code: [Redacted]

Test Article: Modified Sonic Hedgehog Protein (Shh-M)

Sponsor: [Redacted]

Testing Facility: [Redacted]

Proposed Start Date: [Redacted]

Estimated Completion of In-life Phase: [Redacted]

Draft Report to Sponsor: [Redacted]

Protocol Approval

On behalf of

[Redacted]

On behalf of Sponsor

[Redacted]

1.0 STUDY OBJECTIVE

The purpose of this study is to assess the effects of a single supranigral microinjection of Modified Sonic Hedgehog Protein (Shh-M; 1 µg) on the biochemical and functional sequelae of unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal dopamine pathway in rats. Functional assessment will involve testing rotation responses to apomorphine and amphetamine (Ungerstedt and Arbuthnott, 1970). Brain tissue will be removed *post mortem* from all rats to allow for neurochemical and immunocytochemical assessment of the tissue.

[Redacted]

2.0 REGULATORY GUIDELINE

[Redacted]

3.0 QUALITY ASSURANCE

3.1 GLP Compliance

The study will be conducted under the principles of OECD Good Laboratory Practice regulations and guidelines but will not be monitored for compliance by [Redacted]

Exhibit 3

[Redacted]

FINAL REPORT

Title:	Effects of a Single Supranigral Microinjection of Modified Sonic Hedgehog Protein (Shh-M) in Unilateral 6-Hydroxydopamine Lesioned Rats
Project Code:	[Redacted]
Test Article:	Modified Sonic Hedgehog Protein (Shh-M)
Study Director:	[Redacted]
Sponsor:	[Redacted]
Testing Facility:	[Redacted]
Report Prepared by:	[Redacted]
Date of Study Initiation:	[Redacted]
Completion of Experimental Phase:	↓
Date of Report Issue:	

[Redacted]

EFFECTS OF A SINGLE SUPRANIGRAL MICROINJECTION OF MODIFIED SONIC HEDGEHOG PROTEIN (Shh-M) IN UNILATERAL 6-HYDROXYDOPAMINE LESIONED RATS

PROJECT CODE: [Redacted]

We hereby certify that the work reported in this document was carried out by us or under our supervision and represents a true and faithful account of the studies performed.

[Redacted]

[Redacted]

[Redacted]

EFFECTS OF A SINGLE SUPRANIGRAL MICROINJECTION OF MODIFIED SONIC HEDGEHOG PROTEIN (Shh-M) IN UNILATERAL 6-HYDROXYDOPAMINE LESIONED RATS

PROJECT CODE: [Redacted]

This study was conducted under the principles of OECD Principles of Good Laboratory Practice, but was not monitored by our independent Quality Assurance Unit. However, the raw data and report of the study have been subjected to a series of formal quality control procedures within the Department of Pharmacology.

[Redacted]

[Redacted]

TABLE OF CONTENTS

Page